TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		Attorney's Docket Number 02481.1716 U.S. Application No. 09/700391
International Application. No. PCT/EP99/02973	International Filing Date 03 May 1999	Priority Date Claimed 15 May 1998

Title of Invention: Method for Catalysing Complex Reactions of Large Molecules Using Enzymes Which are Bonded to a Polymer Support

Applicants For DO/EO/US: Jürgen BONGS and Johannes MEIWES

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. [ ] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [ ] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [X] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [X] has been transmitted by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [X] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [X] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [X] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [X] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.

16. [X] Other items or information:
    a. [X] Cover Page of WIPO Publication No. WO99/60150
    b. [ ] Copy of Notification of Missing Requirements.

U.S. APPLICATION NO.	INTERNATIONAL APPLICATION NO.	ATTORNEY DOCKET NUMBER
09700391	PCT/EP99/02973	02481.1716

17. [X] The following fees are submitted:	CALCULATIONS
Basic National Fee (37 CFR 1.492(a)(1)-(5)):	
Search Report has been prepared by the EPO or JPO.....$860.00	
International preliminary examination fee paid to USPTO (37 CFR 1.482).....$690.00	
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)).....$760.00	
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....$1,000.00	
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4).....$ 100.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =	**$860.00**
Surcharge of $130.00 for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$

Claims	Number Filed	Number Extra	Rate	
Total Claims	14-20=		X $18.00	$
Independent Claims	3- 3=		X $80.00	$
Multiple dependent claim(s) (if applicable)			+$270.00	$270.00
TOTAL OF ABOVE CALCULATIONS =				**$1130.00**
Reduction by 1/2 for filing by small entity, if applicable.				$
SUBTOTAL =				**$1130.00**
Processing fee of $130.00 for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)).			+	$
TOTAL NATIONAL FEE =				**$1130.000**
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31).		$40.00 per property	+	**$40.00**
TOTAL FEES ENCLOSED =				**$1170.00**
Amount to be refunded				$
charged				$

a. [X] A check in the amount of **$1170.00** to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 06-0916. A duplicate copy of this sheet is enclosed.

The Commissioner is hereby authorized to charge any other fees due under 37 C.F.R. §1.16 or §1.17 during the pendency of this application to our Deposit Account No. 06-0916.

SEND ALL CORRESPONDENCE TO:
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1300 I Street, N.W.
Washington, D.C. 20005-3315
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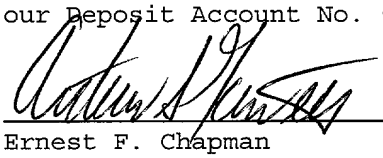


Ernest F. Chapman
Reg. No. 25,961

Submitted: November 14, 2000

PATENT
Attorney Docket No. 02481.1716-00

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re National Stage of International Application No. PCT/EP99/02973 of:	
Jurgen BONGS et al.	
Serial No.: Not yet assigned	Group Art Unit: Not yet assigned
U.S. Filed: Concurrently Herewith	Examiner: Not yet assigned
PCT Filed: May 3, 1999	
For: METHOD FOR CATALYSING COMPLEX REACTIONS OF LARGE MOLECULES USING ENZYMES WHICH ARE BONDED TO A POLYMER SUPPORT	

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

PRELIMINARY AMENDMENT

Prior to the examination of the above application, please amend this application as follows:

IN THE CLAIMS:

Please cancel claims 1 - 10 without prejudice or disclaimer, and insert claims 11 - 21, as follows:

--11. A process for catalysis, comprising:

providing a polymeric support comprising one or more enzymes bonded thereto, wherein the polymeric support material has no pores or substantially no pores; and

catalyzing a complex reaction of large molecules using said polymeric support.

12. A process for enzymatic extraction of biomolecules, comprising:

providing a polymeric support comprising one or more enzymes bonded thereto, wherein the polymeric support material has no pores or substantially no pores; and

extracting said biomolecules from the group consisting of peptides, proteins, oligosaccharides, and polysaccharides.

13. A process for extraction, comprising:

providing a polymeric support comprising one or more enzymes bonded thereto, wherein the polymeric support material has no pores or substantially no pores; and

extracting insulins or their analogs from corresponding precursors.

14. The process as in one of claims 11-13, in which said polymeric support material is a copolymer of the monomers methacrylamide and N,N'-bis(methacrylamide).

15. The process as claimed in claim 14, wherein said polymeric support material has oxirane group-containing monomers.

16. The process as claimed in claim 13, wherein said enzyme is bonded covalently to the support material with the aid of oxirane groups.

17. The process as claimed in claim 13, wherein said enzyme is trypsin.

18. The process as claimed in claim 13, wherein said enzyme immobilized on the support has an activity of .05 to .5 U/ml.

19. The process as claimed in claim 17, wherein said enzyme has an activity of 0.5 to .5 U/ml.

20. The process as claimed in claim 13, wherein the pH of the reaction is 6 to about 10.

21. The process as claimed in claim 20, wherein the pH is in the range of about 7 to about 9.--

REMARKS

Claims 1 - 10 have been canceled without prejudice or disclaimer. New claims 11 - 21 have been added to more particularly point out and distinctly claim that which Applicants consider to be their invention. Support the amendments to each of these claims can be found in the specification and claims of the priority application as originally filed. Accordingly, no issue of new matter is created by these amendments.

If there is any fee due in connection with the filing of this Preliminary Amendment, please charge the fee to our Deposit Account No. 0-6-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

Dated: November 13, 2000

By: _____
Jennifer Swan
Reg. No. 47,118

Description

Process for the catalysis of complex reactions of large molecules by means of enzymes bonded to a polymeric support

The present invention relates to a process for the catalysis of complex reactions of large molecules, namely reactions having undesirable secondary or side reactions, by means of enzymes bonded to a polymeric support and in particular to a process for the enzymatic obtainment of biomolecules, in particular of biologically active peptides, for example of insulins or their analogs, of proteins, oligosaccharides or of polysaccharides, from their biologically inactive precursors, for example preproinsulins, by means of enzymes bonded to a polymeric support.

The preparation of human insulin and its derivatives by means of different approaches is described in the literature. In addition to chemical synthesis, which is uneconomical on account of the complexity of the target molecule, a semisynthetic and a genetic engineering process furthermore exist.

In the semisynthetic approach, a replacement of the C-terminal amino acid of the B chain of porcine insulin catalyzed by trypsin occurs, which leads to the formation of human insulin (H.-D. Jakubke et al., Angew. Chem., 97 (1985) 79).

The genetic engineering approach to the preparation of human insulin proceeds via the stage of preproinsulin and its derivatives, which are likewise subjected to a tryptic cleavage in the work-up (B.H. Frank et al., Peptides: synthesis, structure, function, (1981) 729-738; Jonasson et al., Eur. J. Biochem., 236 2 (1996) 656-661; Kemmler et al., J. Biol. Chem., 246 (1971) 6786-6791).

For efficient utilization of the enzyme, for the semisynthetic approach a process was developed which allows the use of immobilized trypsin (EP 0 294 851).

For the obtainment of insulins or their analogs from the corresponding preproinsulins prepared by genetic engineering, as yet no enzymatic process is known in which the enzyme trypsin is present in immobilized form, so that in known processes, on the one hand, new trypsin must be added for each new reaction batch and, on the other hand, a laborious depletion of the enzyme is necessary in the course of product purification.

The tryptic cleavage of preproinsulin (PPI) is a complex, enzyme-catalyzed reaction with numerous undesirable secondary and side reactions. As shown in Fig. 1, on account of the numerous reactive sites, a large number of reaction products are formed in the tryptic cleavage of PPI, of which the compounds Arg(B31), Arg(B32)-insulin ("di-Arg") and Arg(B31)-insulin ("mono-Arg") are to be regarded as the actual valuable substances for further work-up. Thus, removal both of the presequence and of the "middle" C chain (the C chain arranged in the preproinsulin between the sequence of the A chain and the sequence of the B chain of the insulin) is necessary. If cleavage reactions occur in other sites of the PPI, undesirable by-products are formed, such as, for example, des(B30)-insulin ("des-Thr").

While the cleavage with native trypsin leads to the predominant formation of the two valuable substances, Arg(B31), Arg(B32)-insulin ("di-Arg") and Arg(B31)-insulin ("mono-Arg"), the use of trypsin-immobilizates based on conventional supports such as, for example, Eupergit® C250L, Eupergit® C, Deloxan® leads to an unsatisfactory reaction pattern. The two valuable substances di- and mono-Arg are only formed in small amounts here, while the undesirable secondary or by-products (principally des-Thr) are primarily formed.

It is the object of the present invention to make available a process for the catalysis of complex reactions of large molecules, namely enzyme-catalyzed reactions, in which, as a rule, undesired secondary or side reactions occur, by means of enzymes bonded to a polymeric support, in which the undesired secondary or side reactions are avoided to the greatest extent. In particular, it is an object of the present invention to make available a process for the enzymatic obtainment of biomolecules, preferably of peptides, proteins, oligosaccharides or polysaccharides, from their biologically inactive precursors by means of enzymes bonded to a

polymeric support, in particular a process for the obtainment of insulins or their analogs from the corresponding precursors by means of enzymes bonded to a polymeric support, which leads, with avoidance to the greatest extent of secondary or side reactions, to a selective formation of the biomolecules, in particular of the insulins or of the insulin analogs, and to the associated valuable substances which can be cleaved to give insulins for their analogs.

The object is achieved by a process for the catalysis of complex reactions of large molecules, in which, as a rule, undesired secondary or side reactions occur, by means of enzymes bonded to a polymeric support, wherein the polymeric support material has no or almost no pores which are large enough that the enzymes can bind to the support within these pores.

The object is further achieved by a process for the enzymatic obtainment of biomolecules selected from the group consisting of peptides, proteins, oligosaccharides or polysaccharides, from their precursors by means of one or more enzymes bonded to a polymeric support, wherein the polymeric support material has no or almost no pores which are large enough that the enzymes can bind to the support within these pores.

The process according to the invention is preferably suitable for the obtainment of insulins or their analogs from the corresponding precursors, in particular the preproinsulins, by means of one or more enzymes bonded to a polymeric support.

Insulin analogs are derived from naturally occurring insulins, namely human insulin or animal insulins, for example porcine or bovine insulin, by substitution or absence of at least one naturally occurring amino acid residue and/or addition of at least one amino acid residue to the A and/or B chain of the naturally occurring insulin.

Preferably, the polymeric support material is a copolymer of the monomers methacrylamide and N,N'-bis(methacrylamide), the polymeric support material particularly preferably having oxirane group-containing monomers.

In the process according to the invention, the enzyme is preferably bonded to the support material covalently with the aid of oxirane groups.

In the preferred process for the obtainment of insulins or their analogs from the corresponding precursors, in particular the preproinsulins, the enzyme used is preferably trypsin.

In this case, the enzyme immobilized on the support preferably has an activity of 0.05 to 0.5 U/ml and the pH of the reaction solution is preferably 6 to 10, particularly preferably 7 to 9.

The present invention is illustrated in greater detail below, in particular by means of the examples.

In a number of immobilizations, trypsin was covalently bonded to different support materials (Eupergit® C, Eupergit® 250L, Deloxan®).

The use of these immobilizates in the cleavage of preproinsulin (PPI), however, yielded the desired valuable substances di- or mono-Arg in only a small amount, despite variation of various reaction parameters such as, for example, pH, temperature or enzyme concentration. Instead of this, undesired by-products such as, for example, des-Thr were predominantly formed.

When using trypsin which was immobilized on the support Eupergit® C1Z, a pore-free support, the PPI cleavage surprisingly took place according to the pattern as desired in native use.

The choice of a pore-free support, for example Eupergit® C1Z, in the immobilization of trypsin thus for the first time allows the repeated use of the enzyme with simultaneously very good selectivity in the PPI cleavage in favor of the two valuable substances di- and mono-Arg. A use of the immobilized trypsin analogous to the semisynthetic process with the consequence of easy separability of the catalyst from the reaction solution and of repeated use is thus possible for the genetic engineering process for the preparation of human insulin.

Examples

Example 1 Immobilization of proteins on polymeric supports

Example 1.1 Deloxan®

The immobilization of proteins on Deloxan® requires a prior activation of the support with glutaraldehyde. During the actual attachment of the enzyme, the given native activity of the trypsin was varied in a series of measurements.

Activation:	pH	=	9		[KPP: 100 mM]
	T	=	25	°C	
	t	=	1	h	
	Deloxan®	=	10	g	[VR = 150 ml]
	GD	=	0.2	g/g of TM	

Attachment conditions:	pH	=	9		[KPP: 100 mM]
	T	=	25	°C	
	t	=	3	h	

Example 1.2 Eupergit® C250L and Eupergit® C

In the immobilization of trypsin on Eupergit® supports, the support suspension is mixed with the enzyme solution in a single-stage reaction. Attachment conditions for Eupergit® C250L:

pH	=	8		[KPP: 1 M]
T	=	25	°C	
t	=	3	d	

Attachment conditions for Eupergit® C:

pH	=	8.5		[Borate buffer 100 mM]
T	=	25	°C	
t	=	3	d	
Benzamidine	=	24	mM	

Example 1.3 Eupergit® C1Z

The support Eupergit® C1Z is distinguished in contrast to the other supports by freedom from pores.

Attachment conditions:

pH	=	8		[KPP: 1 M]
T	=	25	°C	
t	=	3	d	
nat. trypsin	=	7400	U/g of TM	[Σ =37,000 U to 5 g]

Under the given conditions, a spec. activity of 405 U/g of TM is achieved. While the attachment yield is comparable to the results of the two other Eupergit® supports, the recovery rate at 46% is clearly higher.

Example 2

A volume of 1 l of the preproinsulin solution having a concentration of 0.83 mg/ml is introduced into a glass beaker. After a pH of 8.3 has been set, the cleavage reaction is started by addition of the trypsin attached to Deloxan® in a concentration of 0.81 U/ml. The temperature of the solution is 6°C over the entire reaction period. The pH is kept constant during the reaction by addition of 1 M NaOH solution. Samples of the reaction solution are withdrawn at regular intervals over a period of 23 h and the content of the individual reaction components is determined by means of HPLC.

As can be seen in Fig. 2, des-Thr is formed as the main product over the period considered, while the amount of the two valuable substances di- and mono-Arg is clearly lower.

Example 3

A volume of 1 l of the preproinsulin solution having a concentration of 0.83 mg/ml is introduced into a glass beaker. After a pH of 8.3 has been set, the cleavage reaction is started by addition of the trypsin attached to

Eupergit® C250L in a concentration of 1.62 U/ml. The temperature of the solution is 6°C over the entire reaction period. The pH is kept constant during the reaction by addition of 1 M NaOH solution. Samples of the reaction solution are withdrawn at regular intervals over a period of 23 h and the content of the individual reaction components is determined by means of HPLC.

As can be seen in Fig. 3, des-Thr is also formed as a main product in this case over the period considered, while the amount of the two valuable substances di- and mono-Arg, which are moreover degraded again in a secondary reaction, is clearly lower.

Example 4

A volume of 1 l of the preproinsulin solution having a concentration of 0.83 mg/ml is introduced into a glass beaker. After a pH of 8.3 has been set, the cleavage reaction is started by addition of the trypsin attached to Eupergit® C1Z in a concentration of 0.081 U/ml. The temperature of the solution is 6°C over the entire reaction period. The pH is kept constant during the reaction by addition of 1 M NaOH solution. Samples of the reaction solution are withdrawn at regular intervals over a period of 23 h and the content of the individual reaction components is determined by means of HPLC.

As can be seen in Fig. 4, the two desired valuable substances di- and mono-Arg are formed in an excess in contrast to the prior reaction experiments with immobilized trypsin based on this support, while the proportion of the by-products (in particular des-Thr) is drastically reduced.

Example 5

A volume of 1 l of the preproinsulin solution having a concentration of 0.83 mg/ml is introduced into a glass beaker. After a pH of 8.3 has been set, the cleavage reaction is started by addition of the trypsin attached to Eupergit® C1Z in a concentration of 0.405 U/ml. The temperature of the solution is 6°C over the entire reaction period. The pH is kept constant during the reaction by addition of 1 M NaOH solution. Samples of the

reaction solution are withdrawn at regular intervals over a period of 12 h and the content of the individual reaction components is determined by means of HPLC. The desired product and by-product spectrum also results in this example on use of the trypsin immobilized on Eupergit® C1Z.

As can be seen in Fig. 5, by quintupling the catalyst concentration the reaction rate can be increased and thus the reaction time needed can be markedly shortened without the selectivity of the reaction thereby being shifted to the disfavor of the two valuable substances.

Possible interpretation of the results of Examples 2 to 5

For example, in the case of preproinsulins (PPI) the starting substrate, in contrast to the secondary products of the reaction, is a larger molecule (≅ 10 kDa), which is subject to a greater pore diffusion limitation. For this reason, the reaction rate of all secondary reactions is greater than the direct reaction of PPI in different fragments. In the case of supports encumbered with pores, for this reason an accumulation of the desired intermediate P (see Fig. 6; in this ex. : di-Arg) should not be possible. The use of pore-free (or almost pore-free) supports therefore offers itself as an approach to the solution of the control of the selectivity.

Legend to figures:

Fig. 1: Reactive sites in the tryptic cleavage of preproinsulin (INS = insulin; A0-Arg-INS = Arg(A0)-insulin; INS-di-Arg = Arg(B31),Arg(B32)-insulin; INS-mono-Arg = Arg(B31)-insulin; des-Thr = des(B30)-insulin)

Fig. 2: Course of the concentrations of the reaction components as a function of time in the cleavage of preproinsulin using trypsin immobilized on Deloxan®

Fig. 3: Course of the concentrations of the reaction components as a function of time in the cleavage of preproinsulin using trypsin immobilized on Eupergit® C250L

Fig. 4: Course of the concentrations of the reaction components as a function of time in the cleavage of preproinsulin using trypsin immobilized on Eupergit® C1Z

Fig. 5: Course of the concentrations of the reaction components as a function of time in the cleavage of preproinsulin using trypsin immobilized on Eupergit® C1Z

Fig. 6: Significance of the support morphology for the selectivity of the reaction (S = substrate, e.g. PPI; P = desired intermediate, e.g. di-Arg; Q = undesired secondary product, e.g. des-Thr)

Patent claims

1. A process for the catalysis of complex reactions of large molecules by means of enzymes bonded to a polymeric support, wherein the polymeric support material has no or almost no pores.

2. A process for the enzymatic obtainment of biomolecules, selected from the group consisting of peptides, proteins, oligosaccharides or polysaccharides, from their precursors by means of one or more enzymes bonded to a polymeric support, wherein the polymeric support material has no or almost no pores.

3. A process for the obtainment of insulins or their analogs from the corresponding precursors by means of one or more enzymes bonded to a polymeric support, wherein the polymeric support material has no or almost no pores.

4. The process as claimed in one of claims 1 to 3, wherein the polymeric support material is a copolymer of the monomers meth-acrylamide and N,N'-bis(methacrylamide).

5. The process as claimed in one or more of claims 1 to 4, wherein the polymeric support material has oxirane group-containing monomers.

6. The process as claimed in one or more of claims 3 to 5, wherein the enzyme is bonded covalently to the support material with the aid of oxirane groups.

7. The process as claimed in one or more of claims 3 to 6, wherein the enzyme is trypsin.

8. The process as claimed in one or more of claims 3 to 7, wherein the enzyme immobilized on the support has an activity of 0.05 to 0.5 U/ml.

9. The process as claimed in one or more of claims 3 to 8, wherein the pH of the reaction solution is 6 to 10.

10. The process as claimed in claim 9, wherein the pH is 7 to 9.

Figure 1

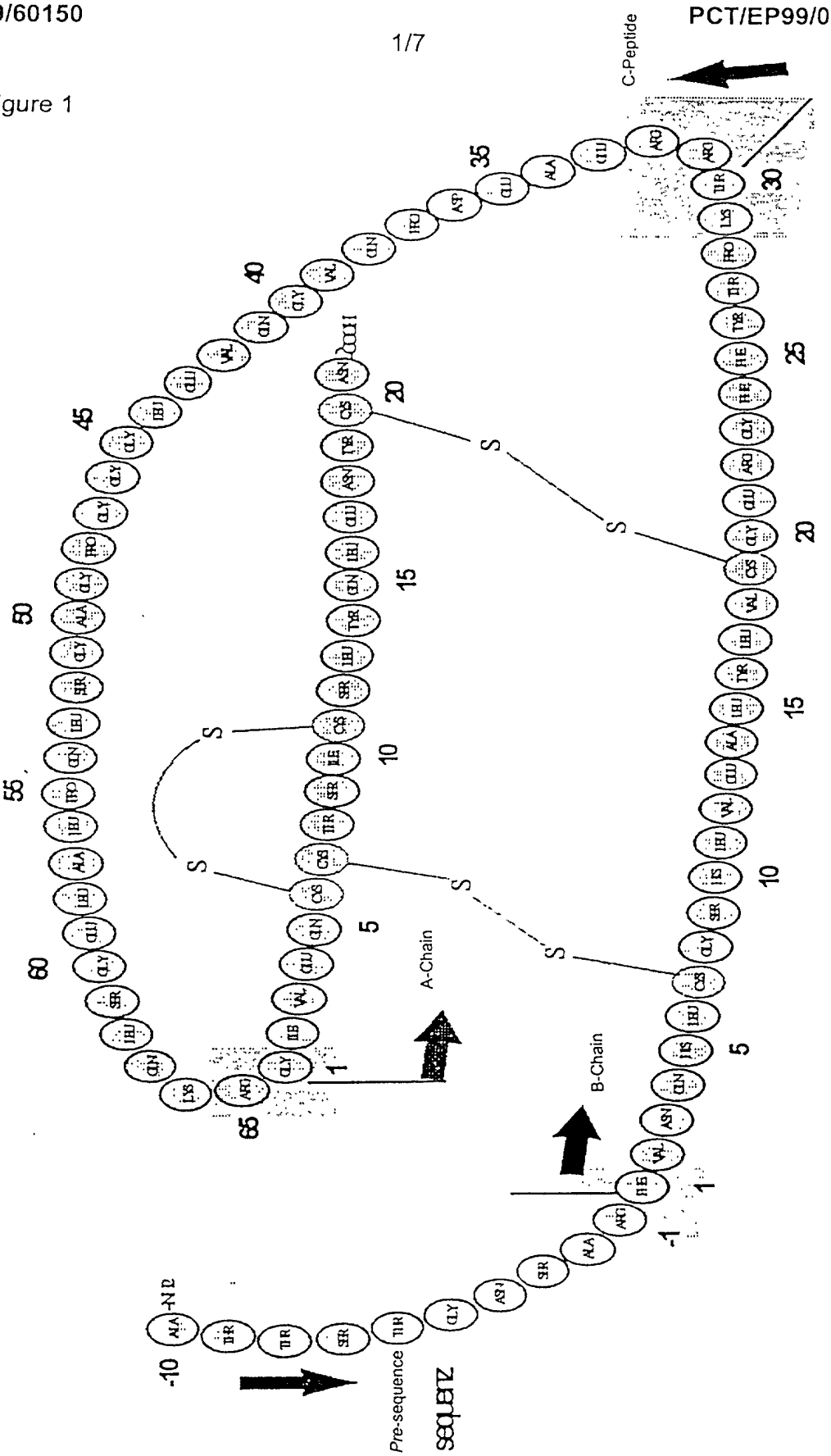


Figure 1 (continuation)

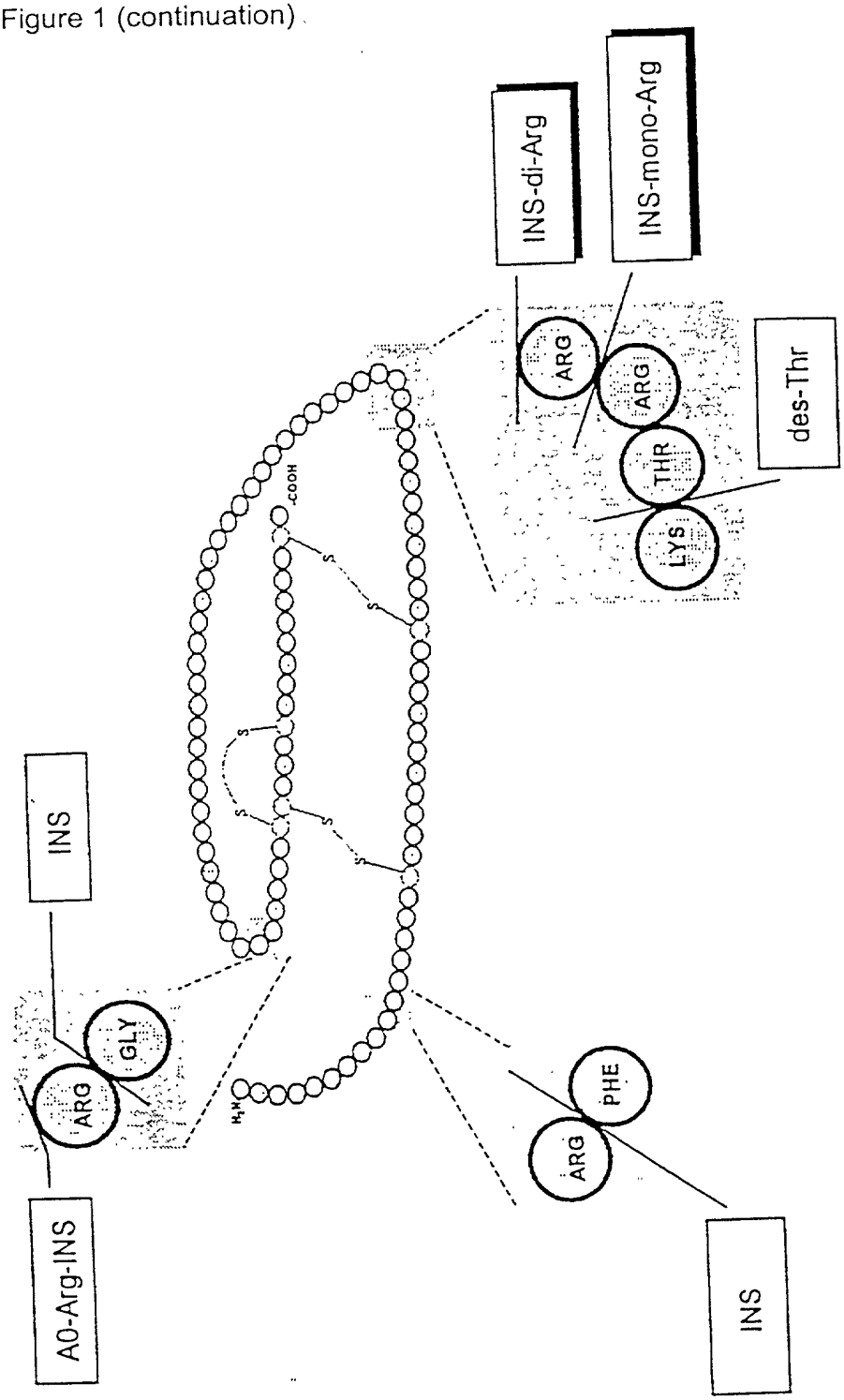


Figure 2

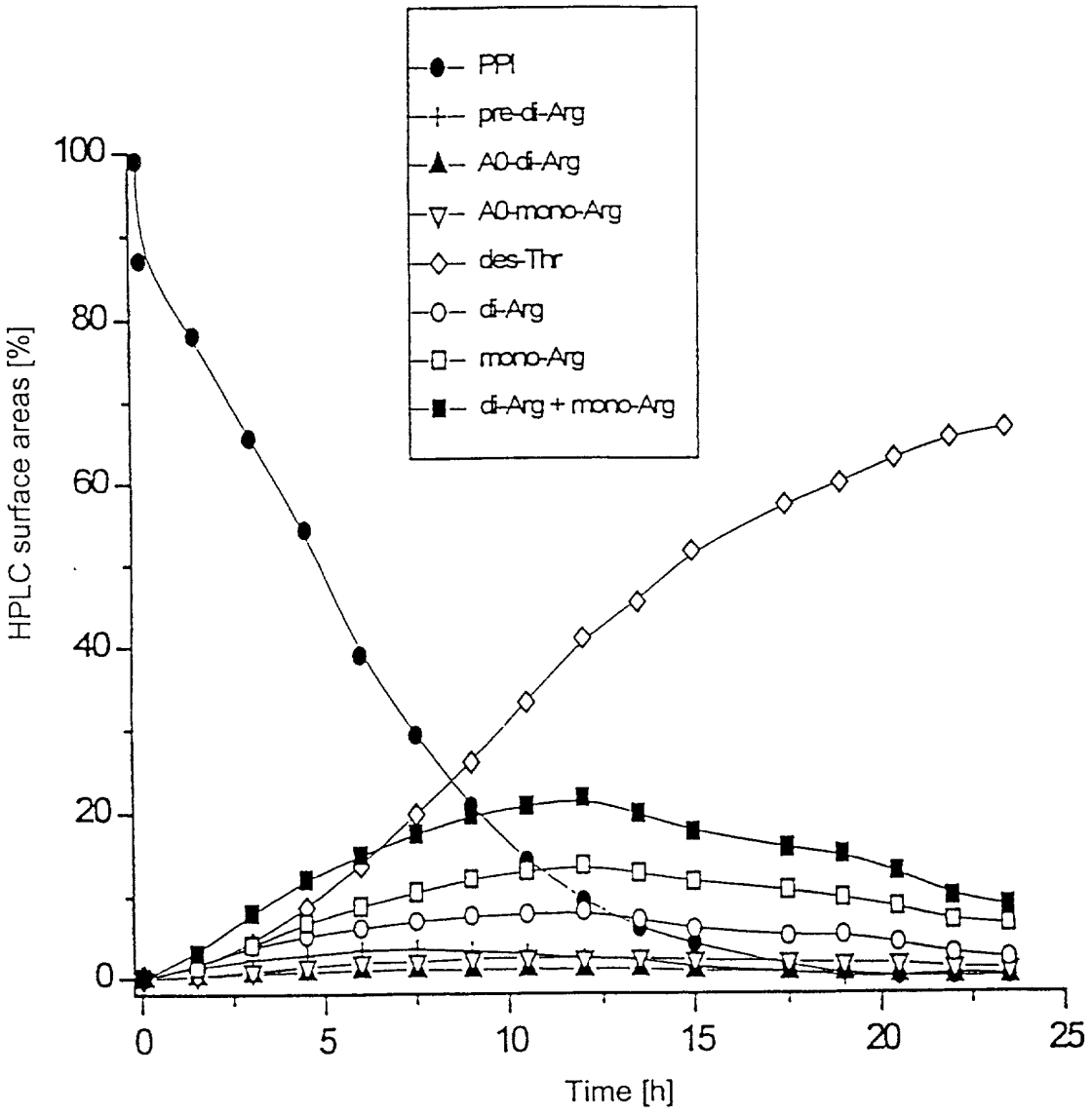

PPI
pre-di-Arg
A0-di-Arg
A0-mono-Arg
des-Thr
di-Arg
mono-Arg
di-Arg + mono-Arg
HPLC surface areas [%]
Time [h]
0
20
40
60
80
100
0
5
10
15
20
25

Figure 3

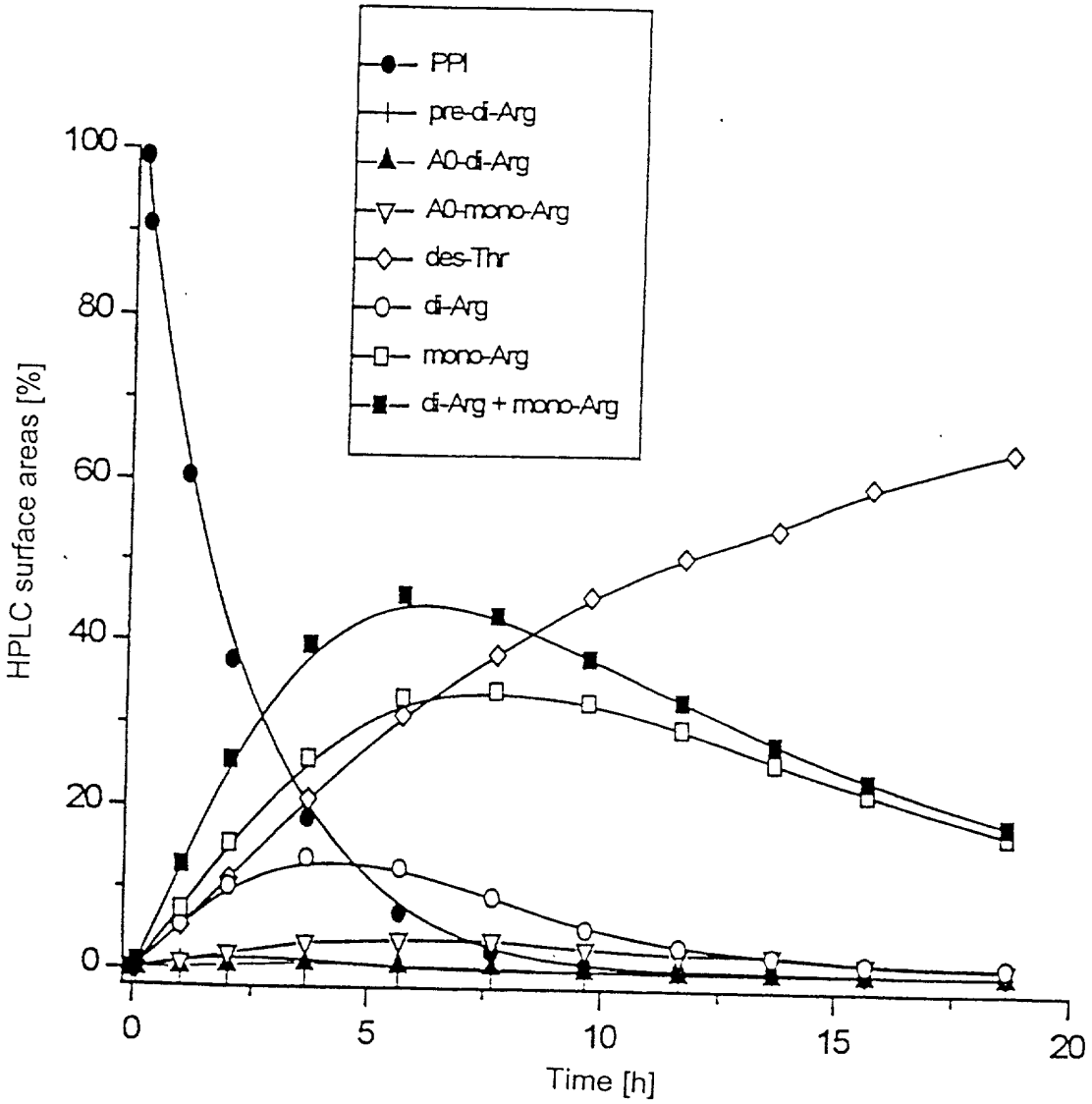
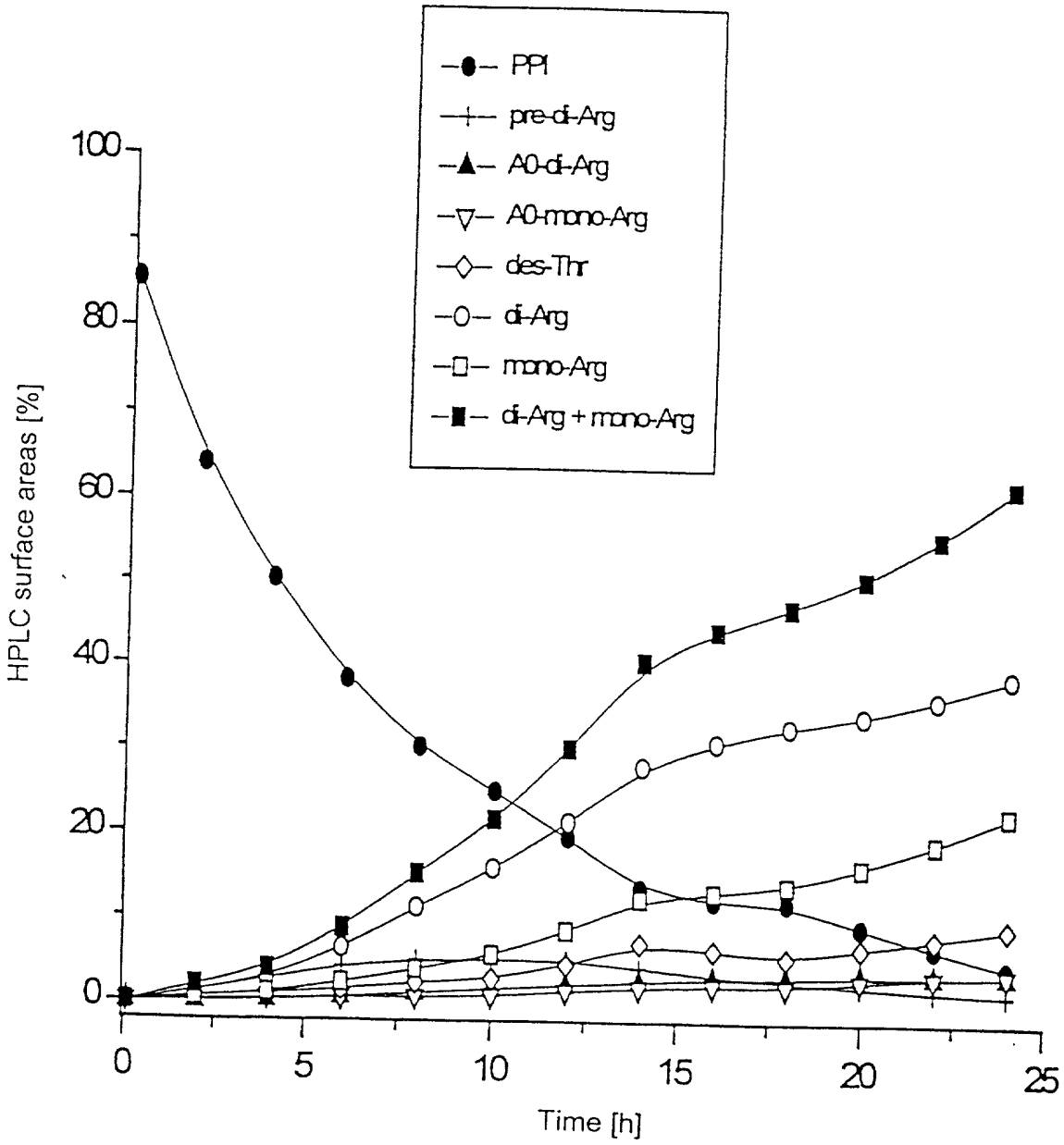

PPI
pre-di-Arg
A0-di-Arg
A0-mono-Arg
des-Thr
di-Arg
mono-Arg
di-Arg + mono-Arg
HPLC surface areas [%]
100
80
60
40
20
0
0
5
10
15
20
Time [h]

Figure 4


PPI
pre-di-Arg
A0-di-Arg
A0-mono-Arg
des-Thr
di-Arg
mono-Arg
di-Arg + mono-Arg
HPLC surface areas [%]
Time [h]
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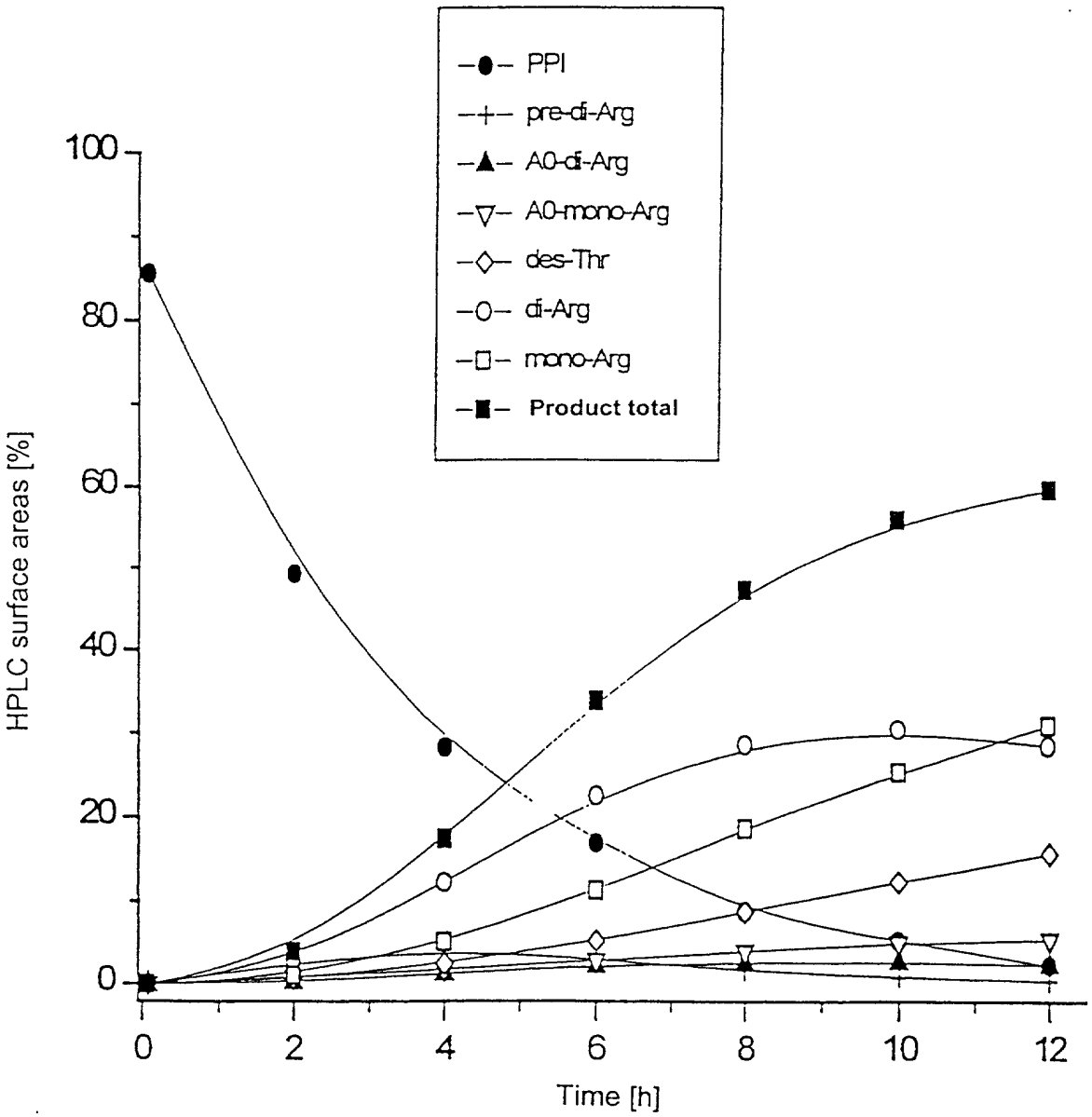
20
40
60
80
100
0
5
10
15
20
25

Figure 5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A0-mono-Arg
des-Thr
di-Arg
mono-Arg
Product total
HPLC surface areas [%]
Time [h]
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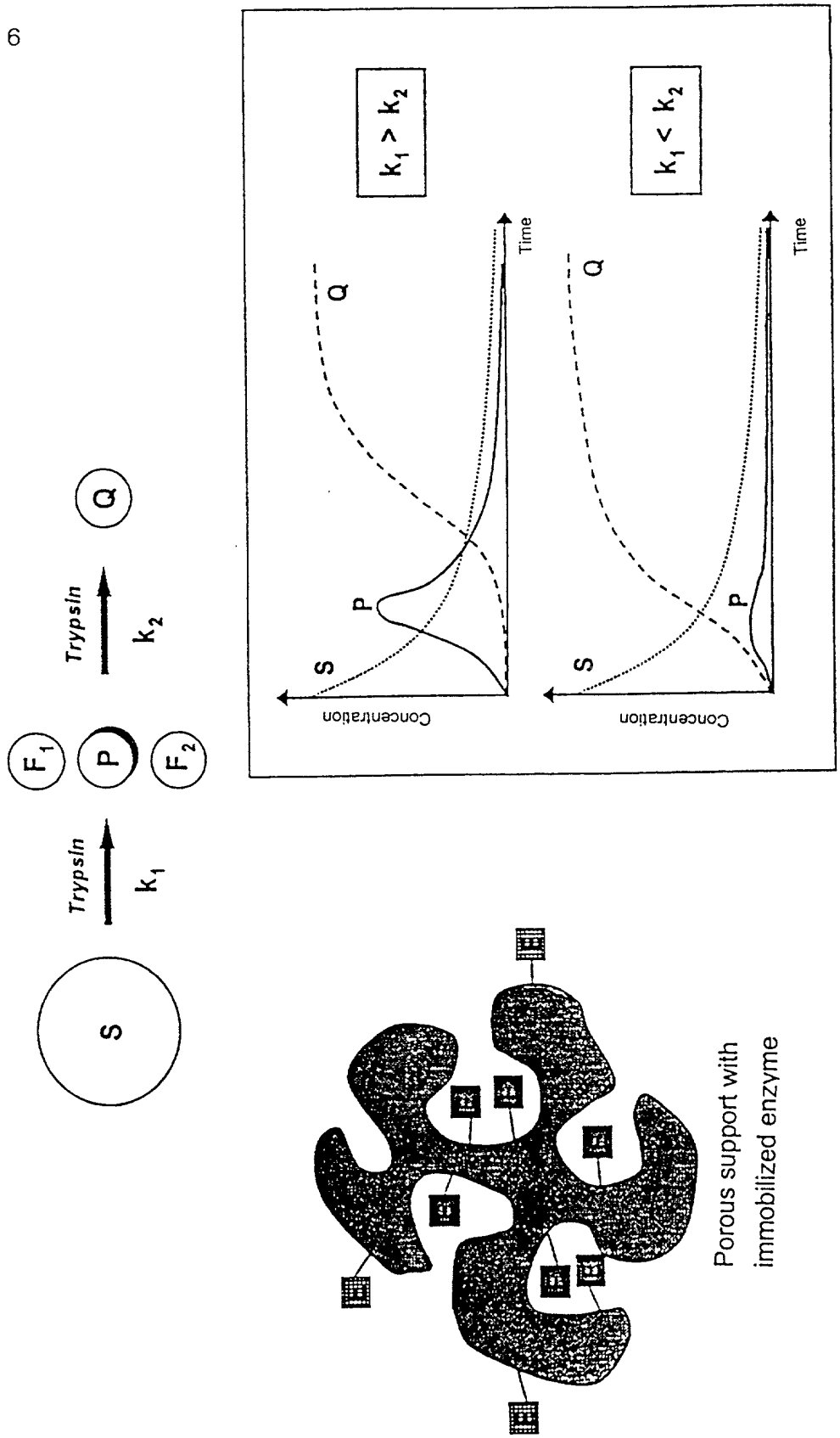
40
60
80
100
0
2
4
6
8
10
12

Figure 6


$k_1 > k_2$
$k_1 < k_2$
Time
Concentration
Q
P
S
Trypsin
k_1
k_2
F_1
F_2
Porous support with immobilized enzyme

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

As below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below, I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

Method for catalysing complex reactions of large molecules using enzymes which are bonded to a polymer support

the specification of which ~~is attached hereto~~ / was filed on May 3, 1999 as International Patent Application PCT/EP99/02973.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s) for which Priority is Claimed:

Federal Republic of Germany, 19821866.4 of MAY 15, 1998

And I hereby appoint
Douglas B. Henderson, Reg.No. 20,291; Arthur S. Garrett, Reg.No. 20,338
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all of the firm of FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, Reg.No. 22,540, my attorneys, with full power of substitution and revocation to prosecute this application, to make alterations and amendments therein, to file continuation and divisional applications thereof, to receive the Patent, and to transact all business in the Patent and Trademark Office and in the Courts in connection therein, and specify that communications about the application are to be directed to the following correspondence address:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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